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Antinociceptive Activity of Methanolic Extract of *Epilobium hirsutum*

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Abstract: Antinociceptive activity of methanolic extract of aerial parts of *Epilobium hirsutum* (*EH*) was determined in the Hot plate and writhing tests in mice. Nearly all extracts showed a dose dependent and marked analgesic activity in mice in the thermal and chemical models of analgesia when compared to the control. Methanol extract at dose of 500 mg kg^{-1} showed higher activity (97.7% writhing inhibition) than diclofenac 50 mg kg^{-1} i.p., (77.8%, $p<0.05$) and morphine 5 mg kg^{-1} i.p. (91.2%, $p<0.05$). Methanol extract, in all tested doses (200-500 mg kg^{-1}) significantly increased the pain threshold in hot plate test ($p<0.05$). EH extract at 200 mg kg^{-1} showed a similar effect to morphine at 5 mg kg^{-1}. These finding indicate the potential therapeutic use of methanolic extract of aerial parts of EH as a potent antinociceptive agent. LD_{50} was obtained 1.5±0.1 g kg^{-1}. EH extract did not induce locomotor impairment in mice at any tested doses.

Key words: Antinocicepive properties, writhing test, hot plate, rotarod, *Epilobium hirsutum*

INTRODUCTION

Various Epilobium species possesses anti-motility activity (Vitali *et al*., 2006) antibacterial activity (Steenkamp *et al*., 2006; Rauha *et al*., 2000), antiinflammatory (Hiermann *et al*., 1991; Juan *et al*., 1988) and analgesic properties (Tita *et al*., 2001). Epilobium spp. used in folk medicine as a treatment for benign prostatic hyperplasia (Vitalone *et al*., 2001; 2003a, b; Steenkamp *et al*., 2006). It seems specifically able to induce the neutral endopeptidase in prostate cancer cells. (Kiss *et al*., 2006a, b, 2004) or inhibits 5 alpha-reductase and aromatase, two enzymes which are involved in the aetiology of benign prostatic hyperplasia (Ducrey *et al*., 1997; Lesuisse *et al*., 1996). One study about decreasing weight of seminal vesicles in mice has published (Hiermann and Bucar, 1997). In addition, it used in Folkloric treatment of tinea capitis (Kilic *et al*., 2001). In spite of many reports about various Epilobium species, there are only limited reports about *E. hirsutum* such as its antimicrobial activity (Battinelli *et al*., 2001; Ivancheva *et al*., 1992) and antitumor activity in mice (Voynova *et al*., 1991).

The analgesic properties of *Epilobium hirsutum* (EH) have not been sufficiently studied so far. Thus, we decided to evaluate the analgesic effect of its methanolic extract, by the classical hot plate and writhing test. EH contains a nearly high amount of flavonoids and polyphenols. There are several papers that suggest biological activity in species of Epilobium is mostly due to presence of different polyphenols and flavonoids (Kiss *et al*., 2004, 2006a; Vitalone *et al*., 2003a,b; Rauha *et al*., 2000; Ivancheva *et al*., 1992; Hiermann *et al*., 1991). In this research, amount of flavonoids and polyphenols were determined as a simple method for standardization of crude extract. Preliminary toxicological study was performed for study of safety of crude extract.

MATERIALS AND METHODS

Chemicals: Quercetin was purchased from Sigma Chemical Co. (St. Louis, USA).
Gallic acid and Folin-Ciocalteu reagent and methanol were purchased from Merk Co. (Germany).

Plant material: *Epilobium hirsutum* (EH) aerial parts were collected from Sari in May, 2005 and confirmed by department of Pharmacognosy. A voucher specimen has been deposited in university's Herbarium (No. 86).

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Materials were dried at room temperature and coarsely ground before extraction. Four hundred gram of dried powdered sample was extracted at room temperature by percolation with methanol (1L×24 h×3). The resulting extract was concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried. For pharmacological studies extracts was prepared in normal salin-tween 80 (95:5).

Animals: Male Swiss albino mice weighing 25-30 g were used for all experiments. They were housed in groups of five under standard light (7.00 to 19.00) and temperature (22±1°C) with food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00-16:00 h). Each animal was used once only. Six mice were used in each experiment

Chemical, pharmacological and toxicity studies: Total phenolic compounds were determined using the Folin–Ciocalteu method (McDonald *et al*., 2001). The contents of phenolic compounds were expressed as gallic acid equivalents (mg g^{-1} of dry mass) from a standard concentration curve. The $AlCl_3$ method (Chang *et al*., 2002) was used for determination of the total flavonoid content of the sample extracts. Flavonoid contents were expressed as mg quercetin equivalent (mg g^{-1} of dry mass) from a standard concentration curve.

The abdominal constriction was induced by i.p. injection of 0.3% acetic acid (10 mL kg^{-1}) following the method of Koster *et al*. (1959). Animals were pretreated with vehicle (3 mL kg^{-1}, i.p.), EH (200, 300, 400, 500 mg kg^{-1} , i.p.) 30 min before the acetic acid injection. Diclofenac (50 mg kg^{-1} i.p.) and morphine (5 mg kg^{-1} , i.p.) were used as the reference drugs. Hot plate test was done according to the method of Eddy and Leimback (1953) in mice. The extract of EH was given at 200, 300, 400 and 500 mg kg^{-1} , i.p. to the animals as a single dose. Effect on motor coordination was assessed using Rota rod apparatus (Harvard, UK) at a rotating speed of 16 rpm (Dunham and Miya, 1957). Rota rod test was carried out in groups of 6 animals after intraperitoneal administration of EH extract at doses of 200, 300, 400 and 500 mg kg^{-1}. Toxicity studies were carried out in mice according to the method of Reddy and Byahatti (1996) and recently published study (Ebrahimzadeh *et al*., 2006). The methanol extract were tested up to 2 g kg^{-1}, in groups of 6 animals, injected intraperitoneally and observed for a week.

Statistical analysis: One-way analysis of variance (ANOVA) for the writhing test or repeated- measures ANOVA (for the hot plate and rotarod tests) followed by Newman-Keuls multiple comparisons test, was used for statistical analysis. Differences with $p<0.05$ were considered significant.

RESULTS

Total phenol compounds, as determined by folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.05x + 0.0545$, $R^2 = 0.987$). The amount of total phenolic compounds in sample was 92.12±2.29 mg g^{-1} of extract powder. The total flavonoid contents of EH are reported as quercetin equivalent by reference to standard curve ($y = 0.0067x + 0.0132$, $R^2 = 0.999$). The total flavonoid content in sample was 58.44±1.38 mg g^{-1} of extract powder. EH extract, in doses of 200-500 mg kg^{-1}, reduced the stretching episodes induced by acetic acid at a dose which was lower than LD_{50}. The effect was dose- dependent and showed a significant effect when compared to the reference. 85.8% inhibition of writhings was obtained by EH extract in 400 mg kg^{-1} . Maximum inhibition of writhings by EH extract was observed at 500 mg kg^{-1} (97.7%). Morphine (5 mg kg^{-1}) inhibited 91.2% of the stretching episodes (Fig. 1). In hot plate test 200 mg kg^{-1} of EH extract showed a significant analgesic activity compared to control group and in the highest dose of EH extract (500 mg kg^{-1}) analgesic activity was higher than that of morphine (5 mg kg^{-1}) (Table 1). We have also tested the analgesic doses of each extract for its effect on motor coordination by rotarod test. EH extract did not produce any significant decrease in the time for which the animals remained on the rotating rod. LD_{50} of intraperitoneal injection of EH extract in animals was obtained 1.5±0.1 g kg^{-1}.

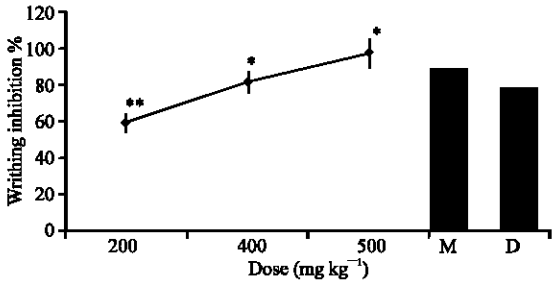

Fig. 1: Writing test. The animals (n = 6) were treated with M (morphine), D (diclofenac) or EH extract. *p<0.05; **p<0.01

Table 1: Effect of methanolic extracts of *Epilobium hirsutum* on hot plate induced nociception in mice

		Response time (sec)					
Treatment	Dose (mg kg^{-1}) i.p.	0	15	30	45	60	75
Control	solvent	6.5±0.4	7.8±1.2	7.2±0.89	6.9±1.5	8±1.4	7.5±0.6
methanolic extract	200	7±0.2	9.7±0.6a	12.5±0.6a	11.55±1.8a	11.2±1.1a	7.7 ±0.6
	300	7.75±2	11.5±0.65a	13.05±1.7a	13±0.68a	9.2±0.3a	8.48±0.49a
	400	4.95±0.77	11.23±2.16a	15.1±1.7a	14.2±3.4a	15.1±1.83a	14.02 ±1.2a
	500	8.48±0.69	18.63±2.5a	21.2±2.65a	19.65±2.4a	17.4±1.48a	16.7±1.35a
Morphine	5	7±0.6	11.5±1.2a	13.1±1.5a	13±0.8a	12.2±1.3a	10.2±1.1a
Morphine	10	6.4±0.8	10.6±1.2a	16.4±1.36a	13.5±0.7a	12.3±0.45a	9.3±0.7a

[a]$p<0.05$ compared with vehicle control, Student's t- test. The response time in seconds was expressed as mean±SEM (n = 6)

DISCUSSION

Epilobium sp. has been used as a treatment for pain, inflammation, adenoma and tumors of prostate in traditional medicine (Tita *et al*., 2001; Vitalone *et al*., 2001; Barakat *et al*., 1997). Also antioxidant and anti-inflammatory activity of this plant can be involved in treatment of the proliferative diseases of prostate. The individuals, who were administered *Epilobium* dried aerial parts tea, claim an analgesic effect from the preparation. Mechanism of analgesic activity of *Epilobium* has not determined yet.

In writhing test, which is more sensitive for non-steroidal analgesics (Tita *et al*., 2001), EH extract in doses of 300, 400, 500 mg kg^{-1} showed 59, 81.85 and 97.67% inhibition of writhings. It decreased the abdominal constrictions in animals significantly comparing to the control group ($p<0.05$). Diclofenac (50 mg kg^{-1}) and morphine (5 mg kg^{-1}) decreased 72.82 and 91.15% of writhings' episodes respectively. Therefore EH extract in 500 mg kg^{-1} showed more analgesic activity than the reference drugs. It seems separation and pharmacological evaluation of the active materials of EH might result potent analgesic substances (Fig. 1). Highest analgesic activity of EH extract in this study was observed in higher amount (500 mg kg^{-1}) comparing with the research reported about *E angustifolium* (380 mg kg^{-1}) (Tita *et al*., 2001). In hot plate test methanolic extract of EH in doses of 200, 300, 400 and 500 mg kg^{-1} showed a faster and longer analgesic effect than morphine at doses of 5 and 10 mg kg^{-1} (Table 1). In this study analgesic activity of *Epilobium hirsutum* in hot plate test was higher than morphine compare to the activity reported for *Epilobium angustifolium* (Tita *et al*., 2001). EH extract in doses with analgesic activity did not show effect on motor coordination that confirms analgesic activity was not due to muscle relaxation and sedation. Half of animals died by EH extract in 1.5±0.1 g kg^{-1} that is to be higher than the analgesic doses. In this study, EH extract antagonized the writhings produced by acetic acid significantly and also decreased markedly the pain caused by hot plate. EH extract showed a higher analgesic activity than standard drugs in this experiment. Finally this study supports EH usage in folk medicine in Iran. Therefore it is useful to fractionate the extracts by different solvents and purify the main active substances in order to evaluate the analgesic effect and probably find some potent compounds.

Of course, various members of genus *Epilobium* containing sterols, triterpene, flavonoids, polyphenolics and specially a macrocyclic (oenothein B) alleviate people's problems through the similar mechanisms (Vitalone *et al*., 2001; Ducrey *et al*., 1997).

According to present study, EH contains almost high level of flavonoid and phenolic compounds. These results might offer a pharmacological explanation for the use of Epilobium in folk medicine. In this study a considerable antinociceptive activity obtained from EH and induces the necessity of the future research in regard to separation and determination of active principles in the extracts.

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